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Previews

NK Cell Genesis: A Trick of the Trail

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In this issue of *Immunity*, Gordon et al. (2012) analyzed the role of the transcription factors T-bet and Eomesodermin in natural killer (NK) cell development, revealing a distinct spatiotemporal requirement of these factors for NK cell maturation.

NK cells are innate lymphocytes that can be cytotoxic and secrete cytokines such as interferon-γ (IFN-γ) (Vivier et al., 2011). NK cells contribute to the control of microbial infections, tumor development, and placentation. NK cells also participate to the shaping of the adaptive immune response (Vivier et al., 2011). NK cell development takes place in the liver during fetal life and in the bone marrow (BM) after birth. The transition from common lymphoid progenitors (CLPs) to NK cells remains to be fully defined.

In this issue of *Immunity*, Gordon et al. (2012) addressed the spatiotemporal involvement of two T-box transcription factors, T-box expressed in T cells (T-bet) and Eomesodermin (Eomes), in mouse NK cell development. They proposed a revised model of NK cell differentiation based on the transient cell surface expression of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and the sequential usage of T-bet and Eomes. Starting from pre-pro NK cells ($CD127^+Id2^{2+}$), which represents the earliest committed NK-cell progenitor, a model of NK cell differentiation based on the sequential and stable acquisition of CD122 (stage 1), NK1.1 (stage 2), NKp46 (stage 3), CD16, NKG2D, DX5 (stage 4), CD11b (stage 5), and KLRG1, CD43 (stage 6) has been recently proposed (Figure 1; Narni-Mancinelli et al., 2011). The report from Gordon et al. (2012) showing that TRAIL is a maturation marker can thus define an intermediate stage of NK cell development, $NK1.1^+NKp46^+TRAIL^+DX5^-T\text{-}bet^+Eomes^-$ (stage 3a) (Figure 1). So far, T-bet and Eomes have been mostly studied in T helper 1 (Th1) cell differentiation of $CD4^+$ T cells, in the acquisition of T cell cytolytic activities, and in the generation of memory lymphocytes. A pioneering work from the Glimcher laboratory has shown that an NK cell-autonomous T-bet deficiency leads to reduced numbers of stage 4 $NK1.1^+$ $DX5^+$ NK cells and no expression of CD43 on NK cells, reflecting the absence of terminally differentiated NK cell population (stage 6) (Townsend et al., 2004). This study also showed that T-bet-deficient NK cells display an increased turnover and an enhanced sensitivity to cell apoptosis that could account for the defect in NK cell number in these mice. Later, T-bet has been recognized to drive the expression of the spingosine-1-phosphate receptor 5 (S1P5) that governs the egress of NK cells from BM and lymph nodes (LN) into the blood (Walzer et al., 2007; Jenne et al., 2009). Eomes, which shares similar functions as T-bet in T cells, is highly expressed in fully differentiated NK cells, but the requirement for Eomes in NK cell development and differentiation was unknown.

Gordon et al. (2012) focused their work on $NK1.1^+NKp46^+TRAIL^+DX5^-$ immature NK cells. TRAIL is known as the dominant cytotoxic effector molecule expressed by NK cells in fetal mice (Takeda et al., 2005). $TRAIL^+$ NK cells have also been described as immature NK cells in the liver of adult mice. Adoptive transfer of either adult liver or neonatal $TRAIL^+$ NK cells resulted in the appearance of $TRAIL^-$ NK cells with a mature phenotype, demonstrating that $TRAIL^+$ NK cells represent an intermediate immature subset that can give rise to mature NK cells. Gordon et al. (2012) confirmed that $TRAIL^+$ NK cells constitute an intermediate stage of NK cell differentiation but also showed the presence of minute amounts of this population in all organs of adult mice. $TRAIL^+$ immature NK cells were present in T-bet-deficient neonate livers but not in T-bet-deficient adult mice. These results suggested that T-bet expression is required for the stabilization of $TRAIL^+$ immature NK cells and that the liver provides an enabling environment for the maintenance of this population as opposed to the BM. $TRAIL^+$ NK cells were lost upon T-bet deletion in NK cells purified from BM or liver further developing into $Rag1^{-/-}Il2rg^{-/-}$ mice.

Eomes-deficient mice exhibited reduced numbers of NK cells. Gordon et al. (2012) showed that expression of DX5 and Eomes correlated with the expression of S1P1 and S1P5. These two S1P receptors are required for an efficient egress of NK cells from the BM and LN (Jenne et al., 2009; Walzer et al., 2007), providing a likely explanation for the reduction in peripheral NK cell counts in Eomes-deficient mice as compared to WT mice. Eomes-deficient NK cells also displayed a cell-autonomous defect in the transition from $TRAIL^+DX5^-$ to $TRAIL^-$ $DX5^+$ NK cells. In addition, Gordon et al. (2012) elegantly showed that the temporal deletion of Eomes in $DX5^+$ NK cells from BM or liver give rise to the accumulation of $TRAIL^+DX5^-$ NK cells, indicating that Eomes is required for the maturation of $TRAIL^+$ NK cells into mature $DX5^+$ NK cells. It still remains to be confirmed whether all mature $DX5^+$ NK cells transit through an immature $TRAIL^+$ stage, because this stage appears to be bypassed in T-bet-deficient mice. Cell fate mapping experiment tracing TRAIL expression will be necessary to resolve this issue. Consistent with the incomplete

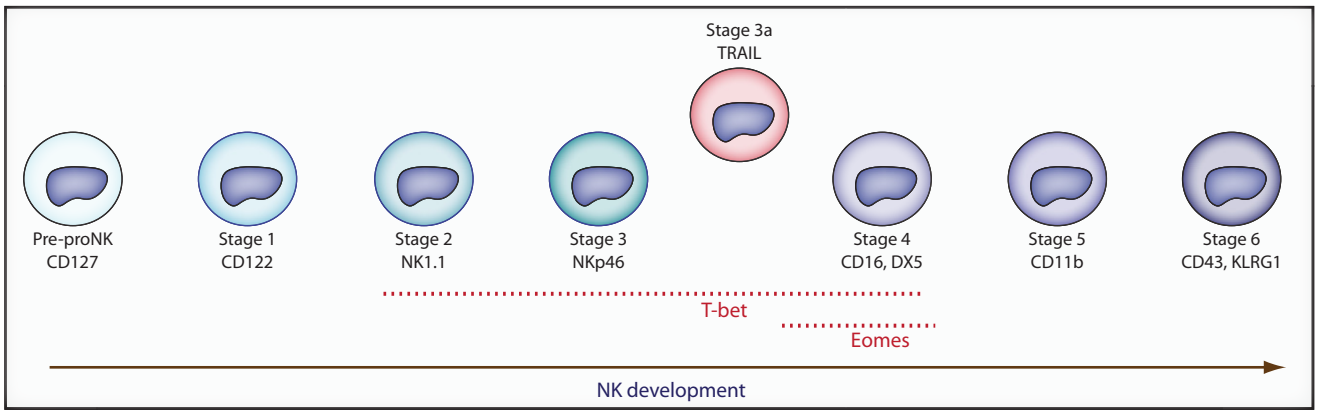

Figure 1. TRAIL, T-bet, and Eomes in NK Cell Development
A model of NK cell development is proposed based on the cell surface expression of indicated molecules. Gordon et al. (2012) showed that T-bet support the NK cell lineage and is required to stabilize the immature TRAIL$^+$ NK cell pool (stage 3a). T-bet and Eomes are later both required for the differentiation in DX5$^+$CD11b$^+$ NK cells. The timeline of T-bet and Eomes actions on NK cell development is indicated by the dashed lines.

NK cell maturation in the absence of Eomes, Eomes-deficient NK cells displayed an altered Ly49 receptor repertoire. Interestingly, the deletion of Eomes in vitro did not alter the expression of the Ly49 molecules, suggesting an epigenetic imprinting on the Ly49 locus.

In the absence of both Eomes and T-bet, NK cells lose the expression of the NK cell lineage markers NK1.1 and NKp46. This striking observation is consistent with the absence of lin$^-$NK1.1$^+$ NK cells in the BM, liver, or spleen of Eomes T-bet double-deficient mice although the number of stage 1 NKPs is not affected. Thus, the absence of NK1.1$^+$NKp46$^+$ NK cells in Eomes T-bet double-deficient mice argue for a redundant but critical role of Eomes and T-bet in the acquisition of NK1.1 and NKp46 markers and further differentiation. Altogether these results suggest that T-bet is the transcription factor involved the stabilization of the immature TRAIL$^+$ NK cells pool. T-bet and Eomes expressions are later both required for the differentiation in DX5$^+$CD11b$^+$ NK cells and progression toward KLRG1$^+$CD43$^+$ NK cells (stage 6). The expression of Eomes alone does not account for the terminal differentiation of NK cells, which is consistent with previous observations showing that GATA-3-deficient NK cells do not fully mature into KLRG1$^+$CD43$^+$ NK cells (Samson et al., 2003). Based on these results, it has been suggested that GATA-3 could regulate T-bet expression in NK cells. It would be thus interesting to determine whether it is also the case for Eomes.

Gordon et al. (2012) also provide insights in the regulation of NK cell effector functions by T-box factors. Eomes$^-$TRAIL$^+$DX5$^-$ WT NK cells are good producers of TNF-α, whereas only a few Eomes$^+$DX5$^+$ NK cells are able to produce this cytokine. In addition, Eomes-deficient and Eomes$^-$ WT NK cells express high amounts of granzyme C and this expression is lost upon maturation into Eomes$^+$ NK cells. In contrast, Eomes$^+$ NK cells express higher amounts of perforin, which is consistent with the role of Eomes at controlling perforin expression in CD8$^+$ T cells (Pearce et al., 2003). Upon phorbol myristate acetate (PMA) + ionomycin or IL-12 + IL-18 stimulation, there is an increased proportion of cells able to produce IFN-γ in the Eomes$^+$ as compared to Eomes$^-$ NK cell population. T-bet expression is not required for this response. T-bet is, however, involved in the expression of granzyme B but not Eomes. Eomes$^+$T-bet$^+$ NK cells are more potent to degranulate as shown by the surface exposition of the degranulation marker CD107a as compared to Eomes$^-$ WT or Eomes$^+$T-bet-deficient NK cells. Interestingly, NK cells temporally deficient for both Eomes and T-bet still express granzyme B, IFN-γ, and CD107a maybe because of the previous imprinting of the T-box transcription factors on these effector genes or more probably because NK cells acquire their effector activities when acquiring the NK cell lineage marker NKp46 (Narni-Mancinelli et al., 2011).

Several transcription factors are involved in NK cell development, including Id2, PU.1, Ets-1, Ikaros, TOX, E4BP4, MEF, GATA-3, and Blimp-1 (Martín-Fontecha et al., 2011). The findings from Gordon et al. (2012) reveal the role of T-bet and Eomes as key checkpoints of NK cell development, although more work is required to precisely determine the precise choreography of all these transcription factor in the shaping of NK cell lineage.

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Fueling Memories

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A hallmark of the adaptive immune response is rapid and robust activation upon rechallenge. In the current issue of *Immunity*, van der Windt et al., (2012) provide an important link between mitochondrial respiratory capacity and the development of CD8$^+$ T cell memory.

At one time, a rite of passage for successfully mastering basic biochemistry was memorizing (at least long enough to recite on an exam) the tricarboxylic acid (TCA) cycle and associated pathways leading to the generation of cellular ATP. The current work of the Pearce lab is forcing us to dust off our old Stryer and/or Lehninger tomes (or is there an App for that?) in order to reexamine the role of these pathways in the development of T cell memory.

Perhaps only rivaled by cancer cells, lymphocyte activation requires an extraordinary amount of energy and biochemical substrates to facilitate expansive cellular division (Fox et al., 2005). Similar to cancer cells, T cells employ aerobic glycolysis as a means of not only generating ATP but also providing substrates for the generation of nucleic acids, fats, and proteins. Indeed, an integral aspect of CD28-mediated costimulation is not only the elaboration of cytokines but also the upregulation of glucose transporters and the activation of biochemical pathways necessary to support these metabolic demands (Frauwirth and Thompson, 2004). Along these lines, more recently, a critical role for *myc* in the upregulation of metabolic machinery necessary for T cell activation has been described (Wang et al., 2011). It was shown that myc-mediated transcription plays a critical role in the upregulation of genes responsible for driving glycolysis but is not essential for fatty acid oxidation (FAO) and increasing the oxygen consumption rate (OCR). Likewise, mammalian target of rapamycin (mTOR) activation, which has been shown to play an important role in regulating CD4$^+$ T effector cell generation, also plays an important role in the expression of proteins involved in glycolysis and glucose uptake (Powell et al., 2011). Thus, it is clear that increases in the metabolic machinery are not simply the consequences of T cell activation but actually play an integral role in promoting T cell activation (Fox et al., 2005). Along these lines, it has been shown that in addition to failing to produce cytokines upon rechallenge, anergic T cells fail to express the metabolic machinery necessary for T cell activation (Zheng et al., 2009). In other words, the upregulation of metabolic programs promotes the activation of T cells, whereas the inhibition of such programs inhibits T cell function.

The initial antigen encounter leads to a massive increase in the frequency of CD8$^+$ effector cells (Araki et al., 2010). Following this expansion there is a contraction phase that ultimately results in the emergence of long-living CD8$^+$ memory T cells with the capability to respond rapidly and robustly upon secondary rechallenge. Thus, memory cells have a unique set of metabolic demands. On the one hand, they must employ pathways that facilitate their long-term survival. On the other hand, they must respond upon rechallenge even more vigorously than naive T cells. Van der Windt et al., (2012) in this issue of *Immunity* sought to determine the role of metabolism in regulating the generation and maintenance of memory cells. Their studies reveal that memory CD8$^+$ T cells possessed a markedly increased mitochondrial spare respiratory capacity (SRC) when compared to effector T cells. SRC refers to the extra mitochondrial ability in a cell to generate energy under conditions of great demand. That is, SRC can be thought of as measuring how close a cell is to its ''bioenergetic limit'' (Nicholls, 2009).

The increase in SRC is dependent upon interleukin-15 (IL-15) signaling, which is already known to play a critical role in the generation of CD8$^+$ T cell memory. The generation of memory CD8$^+$ T cells by exposure to IL-15 concomitantly led to an increase in mitochondrial biogenesis. When compared to CD8$^+$ effector cells, memory cells were shown to have increased mitochondrial membrane potential and less superoxide production,